57. (New) The method of Claim 56 wherein the single nucleotide polymorphism is identified using multiple detector primers, each detector primer comprising a different diagnostic nucleotide.

58. (New) The method of Claim 57 wherein each of the multiple detector primers comprises a different 5' tail sequence.

59. (New) The method of Claim 55 wherein the second primer is an amplification primer.

60. (New) The method of Claim 55 wherein the detector primer comprises a label which becomes detectable upon extension of the detector primer or which produces a change in signal upon extension of the detector primer.

61. (New) The method of Claim 60 wherein the label is a fluorescent donor/quencher dye pair and a decrease in donor dye fluorescence is detected as an indication of the presence or absence of the single nucleotide polymorphism.

62. (New) The method of Claim 55 wherein the diagnostic nucleotide is a 3' terminal nucleotide or about one to four nucleotides from the 3' terminal nucleotide.

Amended Claims: Please amend the claims as follows:

13. (Amended) The method of Claim 1 wherein the second primer is an amplification primer <u>for use in an amplification reaction</u>.

18. (Amended) The method of Claim 1 wherein the presence or absence of the single nucleotide polymorphism is detected by means of a [label associated with the] detector primer <u>which comprises a label</u>.

REMARKS

The foregoing amendments are supported by the specification as filed and therefore do not introduce new matter. Use of strand displacement as the means for separating the detector primer from the target is described at page 6, lines 27-29.

<u>Restriction</u>: Restriction has been required to one of the following inventions: Group I (Claims 1-24), Group II (Claims 25-45) or Group III (Claims 46-54). Applicants provisionally elected Group I with

traverse by telephone on April 10, 2000. The restriction between Group I and Group II is traversed because these inventions are not unrelated. The Examiner alleges that the Group I and Group II inventions are unrelated , but this is a misinterpretation of MPEP §806.04 and MPEP §808.01. The inventions are connected in design in that they employ detector primers which are similar in structure. They are connected in operation and effect because both methods rely on determination of the efficiency of extension of the detector primer to detect SNP's. The method claimed in Group I and Group II are therefore not independent inventions but different embodiments of the same inventive concept. Withdrawal of the restriction between Group I and Group II is therefore requested.

35 USC §112: The amendments to Claims 13 and 18 clarify the language of the claims and overcome the rejection under 35 USC §112, second paragraph. Withdrawal thereof is requested.

35 USC §102: Claims 1-5, 14-18 and 24 are rejected under 35 USC §102(e) as allegedly anticipated by Schram et al. (US Patent No. 5,681,705). For the following reasons, Applicants believe that Schram et al. does not anticipate the claimed invention.

The purpose of the amplification system disclosed by Schram et al. is to detect the various species of the *Mycobacterium avium* complex (MAC) in a single amplification reaction. The Schram et al. amplification system therefore provides "highly efficient amplification of targets in both *M. avium* and *M. intracellulare*" in spite of the fact that "the sequences of *M. avium* and *M. intracellulare* differ by a single nucleotide in the region where the amplification primers hybridize." (emphasis added; col. 4, lns. 58-63) In fact, the Schram et al. amplification primers produce 10^7-fold amplification of both *M. avium* and *M. intracellulare* in spite of the sequence differences between them. (col. 5, lns. 5-10) Each primer of the amplification primer pair described by Schram et al. for detection of MAC forms a single nucleotide mismatch with the target in one of the two species (col. 5, lns. 22-34), however, the observed amplification efficiency is substantially equivalent in the two species. Therefore, the primers described by Schram et al. do not distinguish the sequences difference between the two targets.

In contrast, the claimed invention requires that the SNP be detected based on efficiency of detector primer extension. That is, differences in primer extension efficiency are the basis for being able to detect sequence differences using the methods of the claimed invention. As the primers of Schram et al. result in equal amplification efficiency regardless of the target, they do not detect the presence or absence of the single nucleotide polymorphism and do not contain a diagnostic nucleotide as presently claimed. Schram et al. therefore do not anticipate the claimed invention and withdrawal of the rejection is requested.

35 USC §103: Claims 6-13 are rejected as allegedly obvious over Schram et al. in view of Walker et al. (US Patent No. 5,270,184). As discussed above with respect to the rejection under §102(e), the methods

and primers disclosed by Schram et al. do not distinguish sequence variations between the targets and therefore cannot be used to detect the presence or absence of a single nucleotide polymorphism as presently claimed. The addition of Walker et al. does not overcome this deficiency. *Prima facie* obviousness therefore has not been established and withdrawal of the rejection is requested.

Claims 19-21 are also rejected as allegedly obvious over Schram et al. in view of Walker et al. Again, the Examiner is in error in concluding that Schram et al. teach detecting the presence or absence of a single nucleotide polymorphism. Schram et al. actually teach not detecting the SNP and efficiently amplifying both targets in spite of the sequence variation. Walker et al. does not overcome the failure of Schram et al. to teach detection of the SNP. *Prima facie* obviousness has not been established and withdrawal of the rejection is requested.

Claims 22 and 23 are rejected as allegedly obvious over Schram et al. in view of Thomas et al. Thomas et al. is cited for its teaching of a single nucleotide difference in exon 4 of the HFE gene. This disclosure does not overcome the failure of Schram et al. to teach any method which detects a single nucleotide difference, and withdrawal of thr rejection is requested.

Claims 1-22 and 24 are rejected as allegedly obvious over Vary et al. (US Patent No. 4,851,331) in view of Schram et al. and Walker et al. Vary is characterized as teaching a method for detecting a single nucleotide difference, but failing to teach displacing the primer extension product by extension of a second primer. The teaching of strand displacement is allegedly provided by Schram et al. At pg. 7 of Applicants' specification, beginning at line 14, the uncertainties associated with using strand displacement in isothermal SNP detection systems are discussed in the context of the variable results obtained with amplification primers and signal primers. In summary, prior to the present invention it was not known whether or not using strand displacement to separate the extended detector would allow detection of the SNP. This is because extension of the upstream primer and strand displacement are isothermal enzymatic reactions which occur at a predetermined rate not readily controlled by the practitioner. If strand displacement occurs too slowly relative to extension of a mismatched detector (allowing the detector sufficient time to be extended in spite of the mismatch) or if strand displacement occurs too rapidly relative to extension of a matched detector (causing it to be displaced before extension proceeds sufficiently to be detected) a false result will be obtained. In contrast, thermocycling in PCR allows the practitioner to control the amount of time allowed for primer extension and to adjust it to obtain optimum discrimination of sequence variations. With respect to isothermal extension and displacement reactions, it was not known prior to the present invention whether or not strand displacement would occur at a rate appropriate to allow detection of a sequence variation using a downstream detector primer.

Schram et al. is relied upon for its teaching of strand displacement, which is to be combined with the methods and probes disclosed by Vary et al. However, Schram et al. fail to show that such strand displacement methods can be used successfully to distinguish sequence variations. In fact, the sequence

variations in the targets of Schram et al. are clearly not distinguished using the strand displacing methods described. The combination of references therefore does not provide any motivation to make the asserted combination. In fact, by suggesting that a strand displacing approach would not be successful for detecting SNPs Schram et al. teach away from such a combination of methods. *Prima facie* obviousness therefore has not been established and withdrawal of the rejection is requested.

Claims 22 and 23 are rejected as allegedly obvious over Vary et al. and Schram et al. in view of Thomas et al. The foregoing discussions of Vary et al. and Schram et al. apply here as well, and the basis of the rejection fails to establish a case of *prima facie* obviousness. Withdrawal of the rejection is therefore requested.

Double Patenting: Claims 1-8, 11, 12, 15-18 and 24 are rejected for alleged obviousness-type double patenting over Claims 3, 7, 10 and 13 of Schram et al. The claims of Schram et al. recite SEQ ID NO:1 and SEQ ID NO:2, which in spite of sequence mismatches with different MAC species do not detect sequence variations in the methods claimed. The claims therefore cannot be construed to teach or suggest detecting an SNP based on a determination of primer extension efficiency. The presently claimed methods, which recite detection of a sequence variation based on a determination of primer extension efficiency, are neither taught or suggested by the claims of the reference. The double patenting rejection is therefore improper and withdrawal is requested.

CONCLUSION

In view of the foregoing amendments and remarks, Applicants' respectfully submit that the present application is in condition for allowance. An action passing this case to issue is requested. If the Examiner is of the opinion that a telephone interview would be useful to resolve any outstanding issues in this case, she is invited to contact the undersigned at the number shown below.

Respectfully submitted,

Donna R. Fugit

Donna R. Fugit, Ph.D.
Agent for Applicant(s)
Registration No. 32,135

BECTON, DICKINSON AND COMPANY
1 Becton Drive
Franklin Lakes, NJ 07417
(201) 847-7166
(201) 848-9228